COLLAGEN SHEET AND AMNIOTIC MEMBRANE APPLICATION OVER SUPERFICIAL BURN—A COMPARATIVE STUDY

THESIS

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INTRODUCTION

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Fire is one of the earliest friends of mankind. It is a necessity of life. It has also caused great suffering to mankind too. The thermal injuries are very old. With the advancement of science, modes and quantum of thermal injuries have increased tremendously. In India itself one lac and ten thousands deaths are reported each year due to thermal injuries (Sinha 1968). Out of which 1200 deaths occur in Bombay alone. Approximately 3000 persons are severely burnt every year in Bombay city and of these 74% are domestic burns and 79% of all domestic burns involve women and children.

Various modes of treatment were suggested and used from time to time yet it has to gain perfection. In ancient days the emphasis in treatment of burn wounds was local application of various medicinal products like resins and Bitumen, vinegar, extracts of plants, honey and bran, gum, Goat's hair and other funny things as milk from a mother who gave the birth to a male baby. Subsequently these local applicants changed to chemicals as tannic acid, silver nitrate, gention violet and petroleum gauze. With the advent of antimicrobials the emphasis turned to antimicrobials as local applicants as mefimide or sulfamylon, silver sulfadiazine, cerium

nitrate and other antibiotics as genticyn and soframycin.

In spite of the local treatment mortality and morbidity remained almost unchanged which led to the appreciation of basic concept of burn injuries in the form of metabolic changes in the body secondary to discharge and infection of raw burn area. There is excessive loss of body constituents e.g. water, minearls, proteins and secondary infection due to invasion by micro organism. This infection interferes with and delays the normal healing process resulting in deformities, contractures cicatrices and scars. Other problems include denution of nerve endings causing pain leading to restriction of movement ultimately resulting in contractures.

Hence present day emphasis in the management of burn, is on covering the raw area. A superficial burn e.g. with many of the deep epithelial cells preserved has the potential for good healing within two weeks.

The coverage of the uninfected raw area with autograft is ideal however, the availability of autograft particularly when the burn area is large is rather limited or at times not possible for various reasons. The next best solution is the coverage of

burn area with homograft. Here again the availability is limited. Various other biodressings and synthetic materials have been used by different workers. The biodressings include cadaver skin, porcine skin, foetal membranes (amnion and/or chorion), synthetic skin and collagen sheets. The synthetic materials include various fibres, foams, fabrics, sprays, gels and laminates.

In the present work the burn surface has been covered with two different biodressings, i.e. human amniotic membrane and collagen sheet. At the same time a comparative study is made to assess the superiority of either of them.

REVIEW OF LITERATURE

REVIW OF LITERATURE

Thermal injury presents a therapeutic challange to the Surgeon due to its immediate altered anatomy & physiological derangement. It is a catastrophic illness causing overwhelming insult to the patient's psychological aspect and at the same time involves the family in a great financial loss.

Burns are caused by ppplication of heat to the body. The depth of resulting burn injury will depend on intensity and duration of heat application and the conduction of tissue, involved. Thermal injury causes no damage below 45° C of temperature. All gradations of cell injury occur between 45-50°C temperature and the denaturation of proteins become apparent above 50°C.

The management of burn patient comprises of two parts (1) General management comprising of maintenance of airway, intravenous resuscitation, sedation, tetanus prophylaxis and administration of systemic antibiotics. (2) Management of local wounds - The present day excercise is the outcome of ideas, observations and experiences of various workers. There are two main methods. Exposure method - The principle of t is method is that drying the area of the burn inhibits the growth of bacteria and ultraviolet sunlight is hostile to bacterial

growth, eventually a dry surface is obtained and topical agents may be applied as a further deterrent to bacterial growth.

Close Method - The underlying principle of this method is the fact that the majority of burn are sterile or have no pathogenic organisms on their surface in the first few hours from the time of burn. If the area can be completely sealed off from its surroundings by means of sterile dressing.

There is wide range of dressing material and one finds it very difficult to choose ideal material. This leads to definition for good dressing as following:-

1. It must be readily available material.
2. It must have no antigenic properties.
3. It must be sufficiently strong and flexible.
4. It must be adequate to cover the wound and isolate it from the enviornment.
5. It must be capable of being sterilized.
6. It must have water vapour transmission rate which will allow the proper moisture balance in the repairing wound.

The Egyptians treated the burn by incantation and a mixture of gum, goat's hair and milk of a mother who had given birth to a son, around 5th-6th

centuries B.C.. Around 430 B.C., Chinese and Japanese used tinctures and extracts from tea leaves. Hippcrates, the father of Medicine, used resins and Bitumen mixed with melted swine seam, spread over a piece of cloth on the burn surface after warming it. He also used warm vinegar soaked dressing to relieve the pain. For the scar of burn he used solution of oak bark for tanning. In ancient Rome Celsus suggested mixture of honey and bran for local application. While Pliny and Elder advocated exposure method for the treatment of burn. Galen applied vinegar or wine locally over burn surface.

Paulus of Aegina (7th century A.D.) used various emolient preparations. The famous Arabian physician Rhazes used ice cold water locally in 9th century. Ambrose Pare (1517-1590) suggested ointments for the treatment of burn wound. Clowes (1991) used different complex preparations on the different parts of body area involved in burn. Edward kentish (1797) advocated pressure bandage to relieve the pain and to prevent blister formation. Marjolin (1797) described that certain scars of burn wound changed to be malignant and they were termed after his name i.e. Marzolin ulcer. H.Earies (1799) suggested the use of ice cold water and described it as a good analgesic.

Guilhelmus Febricus Hiddemus (1607), the

father of German Surgery, described three degrees of burns according to depth. L. Heister (1682-1758) classified burn into 4 degrees. Beron Gullaume Depuytren (1832) a famous French Surgeon, classified depth of burn in 6 degrees and described four stages in the disease process of burn. (1) Period of irritation (2) Period of inflammation (3) Period of suppuration (4) Period of exhaustion. He also told about gastro intestinal haemorrhage in the cases of burn.

In the 19th century Syme (1827) suggested dry cotton wool dressing with firm pressure to cover the raw area. For gastrointestinal haemorrhage Curling (1842) recognized gastric and duodenal ulcers as a cause. Passwant suggested saline bath for burn area. Copeland (1877) advocated exposure method for burn area.

In the present century Edward Clark Davitson (1894-1933), at Henry Ford Hospital, suggested the use of tannic acid on burn surface in 1925. He claimed that these agents decrease the fluid loss, relieve pain and produce a clean scar. Later on Mcclure, of the same hospital, in 1944, described it as hepatotoxic and attributed many deaths to this toxicity. Aldridge (1933) advised the use of gention violet as escharotic agent on burn surface. Other escharotic agents as .5% $AgNo_3$ and a long list came into light. The use of these agents persisted till the begining of world war II.

Allen and Kock of Chicago (1942) suggested the use of petroleum gauze piece locally with strict immobilisation. These types of occlusive dressings were in use in army hospitals during world war II.

Wallace of Edinburgh (1949) in England and Pulaki, Artz and Blocker (1950) in U.S.A. reintroduced exposure method for burn wound. Later on other surgeons accepted the same method with the view that development of crust provides physiological covering to burn wound, thus reducing the effect of raw area.

Leidbug, Reiss and Artz (1953) attributed primary cause of death of septicemia and staphylococci to be the main microbial. As antibiotics against Gram positive organism developed, the sppsis due to pseudomonas became very common and cause of death was attributed to it. It was because of Gram negative organism and other microbials that a great importance was laid on to develop the antimicrobial agents which can penetrate the burn surface and minimize the growth of such microbials. Various such antimicrobials, e.g. .5% silvernitrate (Moyer), Mefemide or sulfamylon (Moncrief), silver sulfadiazine (Fox C.L.Jr., Rappole B.W., Stenford W., 1969), Cerium Nitrate (Williams W. Monofo, Som N. Wandon), Cerium Nitrate and Silver Sulfadiazine (Fox. C.L.Jr.,1975) were tried and are

still in use. But these topical agents are effective merely in controlling microbial population. It is stated that from an average of 10^7 organism per gram of tissue it is reduced to 10^4 per gram of tissue by these agents (Artz C.P.).

BIO-DRESSINGS

Homografts, autografts, and hetrografts have been tried for many centuries. In 1881 Girdner treated a lightening burn with skin from a suicide victim. In the same year Shede used skin from freshly amputated specimen as well as from cadaver to cover open wounds.

In the middle of the present century (1952) Dogo of Italy noted the usefulness of cadaver skin obtained in viable state.It was useful where it was possible to preserve the tissue till the time of application. He measured skin viability by determining the tissue oxygen uptake in the Warburg apparatus. The skin was preserved at 3°C in physiological solution. The oxygen consumption of cadaver skin was noted unaffected upto 16 hours after death.

The biological skin dressings as life saving measures in the treatment of extensively burnt patients were popularized by Brown in 1952. His work

was extended in 1953 in a report dealing with the use of postmortem allografts as biological dressing. Brown stated that skin may be recovered even days after death from cadaver if placed in cold storage. These grafts have been used for number of years and are still in used as life saving measures to cover extensive areas where autograft are not available and in emergencies.

Eade(1958) and Morries (1966) observed that the homografts had antibacterial property. They pointed out about the decrease in count of bacteria within 2 hours of homograft application. More recently homografts have been applied to prepare burn wound for autograft (Miller 1967).

In the 1960's skin from dogs and pigs came into use. Porcine heterografts are now popular as temporary coverage for granulating wound. Miller et al (1967) showed normal architecture with recognizable basal layer and normal collagen bundles in the dermis, if healing had taken place under the coverage of homografts.

Sharma et al (1978) used the homografts in 25 cases of which 15 were superficial burns. There homografts were donated by voluntary donors. These grafts were preserved in a solution containing plasma, normal saline, antibiotics and phenol red at the temperature of 0-4°C. The survival period of these grafts in the

cases of similar blood group to that of donor, was 11 days while it was 13 days where the blood of the patient was not of the same group as that of the donor.

Allografts skin, have their limitations though they fulfill most of the criteria for satisfactory biological dressing. Baxter (1970) estimated rise in physician hours and hospital cost of $ 225 per patient as the cost for cadaver allografts.

Heterografts (Xenografts)-

In 1960, canine skin had been used by Switzer et al in the treatment of thermal injury. Porcine skin is the material of chbice for xenograft. Bromberg et al and Elliott and Hoehm used pig skin as a temporary bio-dressing. Salisbury et al reported poor results of dressing of these xenograft on donor sites. Comparative experiments were done of exposure treatment with covering by fine mesh gauze.

Amniotic Membrane -

The amnion is the inner foetal membrane. It's inner surface is in contact of amniotic fluid and foetus. It's outer surface is separated from decidua of uterus by chorion. It has following parts -

(1) Placental amnion - lines the inner aspect of placenta.
(2) Reflected amnion - lines rest of the chorion.
(3) Dependent amnion - overlies the internal os of

Histologically it has five layers:

1. Epithelium-It is composed of a single layer of apparently simple, non ciliated cuboidal cells. Recently it has been suggested that amniotic epethelium has a role in exchange of fluid and electrolytes between amniotic sac and mother.

2. Basement membrane-It is narrow band of reticular tissue below the epithelium and is firmly adhered to it.

3. Compact layer- It is dense, a-cellular layer, immediately below the basement membrane and is firmly adhered to it.

4. Fibroblast layer - It is composed of fibroblast net work in the mesh of reticulum having fibroblast and Hoflour cells (Macrophages).

5. Spongy layer - It is composed of extra embryonic coelomic reticulum. It is capable of great distension and contains mucus.

The thickness of membrane is variable due to variable amount of mucin and fluid in spongy layer. Normally the thickness is 1/50 to 1/2 mm. which can increase as much as 2-5 cms.

Blood Supply - The amnion does not have any blood supply at any time of gestation.

Nerve Supply- The nerve supply to amnion has not been confirmed.

Lymphatic Drianage - Some workers gave the possibility of presence of lymphatic vessels in amnion. The many large spaces are present between the bundles of the reticular fibres of fibroblast and spongy layer but the presence of actual lymphatic vessels have not been confirmed.

Embryology - The development of amnion begins during transformation of embryo from morula to blastocyst stage about 7-8 days after firtilization. At the periphery of ectodermal layer of polyhedral cells, some cells are separated from inner cell mass. These cells are termed as ammniogenic cells, and they form a slit like cavity with the appearance of primary extra embryonic mesoderm. The amniotic epithelium becomes separated from primitive trophoblast. Amniotic mesenchyme is derived from the primary extra embryonic mesoderm of the blastocyst.

Immunology- There are various studies reported to observe the facts concerning this aspect. It was observed that amnion was taken up as permanent graft when implanted to its own new born infant. The revascularization was not seen. The nutrition of graft appeared to be by simple diffusion. When allograft amnion was implented subcutaneously,the results were similar to that of autograft for first 14-17 days.

Later on these allografts were changed in to hyalinised substances. Only mild round cell infiltration was observed after 20-30 days. Similar results have been observed of allografts and homografts as surface bio-dressing. It was observed, when mesenchymal surface was placed towards the host, the 'take' or 'fixation' was superior and when amnion was placed towards the host, little fixation was noticed at the end of 72 hours. In any case neovascularization was not observed.

When allograft amniotic membrane was applied in pelvic cavity after pelvic exenteration, it was recovered after 3 weeks and was histologically viable. It was observed that fibroblastic activity was markedly inhibited as compared to that of control case.

In an experimental study amnion allografts, when implanted intra peritoneally, prevented adhesion formation and the gradual disintegration of graft without any host responce. These evidences suggest the low antigenicity of amnion. No violent host reaction is noted yet.

Clinical and Experimental Application -

First person to report the attempts of grafting pieces of lining of amniotic sac on granulating wound, was John Staigu Davis in 1910 at John Hopkins University.

Sabella (1913) used amniotic membrane on raw surface caused by burn and ulceration. He suggested amnion side to be kept exteriorly because of its ectodermal origin. They observed reduced pain, rapid reepithelization and absence of infection.

Davis (1919) observed these dressings to be eventually rejected.

Brindeau (1935) and Burger (1937) reportedd the use of amnion graft in construction of artificial vagina. He later used amnion successfully in the repair of experimental defects in rabits, dogs and cats. De Roth (1940) reported successfully that amnion was used in conjuctival repair.

Chao et al (1940) used amnioplastin prepared from amniotic membrane to prevent adhesions following the craniotomy. Amnioplastin was prepared by keeping the amniotic membrane in 70% alcohol. Then it was washed in water and was dried in air. He applied it over lacerated piameter. No adhesions were observed in any case. The amnioplastin gradulally disappeared and mucoid material was observed as the remanent after 10th day and there was slight evidence of mucoid material after 20th day. There was no evidence after 30th day. Histologically no evidence of foreign body reaction and organised adhesion were observed after 10 days. The

growth of fibroblast beneath and above the membrane was the only reaction to disintegrating foreign body. The remaining amnioplastin was as amorphous substance without cellular differentiation. There was no evidence of material after 30th day and defect in piameter was completely filled.

Kubanyi (1941-48) reported the use of amnion in burn, traumatic skin loss and to prevent intra abdominal adhesions. He also tried amnion successfully in the repair of enterocutaneous fistulae in one case. He suggested the further use of amnion in prevention of adhesions.

Pinkerton (1942) reported the use of amnioplastin to prevent the adhesions between flexor tendons and their sheaths. He found amnioplastin as gelatenous transparent membrane after 3 months of application.

Hensen (1950) used amniotic membrane in the management of non healing ulcer of skin. He compared the granulation tissue beneath amniotic membrane to that of other methods as plaster of Paris coverage.

Douglas (1952-54) used homografts of foetal membrane as bio-dressings over burn area in 1952. He showed the effect of use of hetero-homograft membranes and homograft skin in an experimental study

in 1954. He also used human placental membrane on chorio-allantoic membrane of cheek. He observed the mean survival time of chorion allograft to be 13 days. He noticed the time taken for reepitheliazation to be 14 days in chorion covered wound areas. He showed the neovascularization in grafted chorion as a sign of graft take up. He stated human chorion as graft to be better than alloplastic skin grafts. By tissue chamber technique he observed that plasmic and haemic circulation remained active. The fissures were developed at surface but cells remained actively growing in perimeter of membrane transplant.

Jullian A. Sterling (1956) successfully used the amniotic membrane over old infected flame burns. He advocated it's dressing as emergency measure in trauma.

Pigeon (1960) observed following effects in burn cases dressed with amniotic membrane:

(A) Immediate Effects -

1. Pain relieved at once after application and no further analgesics were required.
2. Antibiotics were used only after development of complication.
3. The dressings were generally found dry.
4. Healing of wound was rapid and complete.

(B) Delayed Effects -

1. No discolouration was observed in amnion treated cases.

2. Minimal scar tissue formation.
3. No contracture was observed in amnion treated cases.

He also stated that amniotic membrane undergoes changes similar to that in cornified cells.

Masses and collagues (1962) in an experimental study in dogs, used foetal membrane to replace parietal peritonium after pelvic exentration. After 59 days very few adhesions and dense scar tissue were found. Human trials failed at that time.

Dino (1965) used the amniotic membrane in burn cases and favoured it in comparison to control cases on following points -

(1) It is homograft, closely resembling the skin being a direct continuation of foetal integument along the umblical cord.
(2) It is easily available with negligeable cost.
(3) It is fairly strong and stretchable to cover a side area.
(4) It has minimal contact with maternal blood.

Dino (1966) found the best preservative for amniotic membrane. He preserved amniotic membrane in following solutions:- 1. Sterile normal saline solution. 2. Bensal konium chloride (1:1000 dilution) in sterile saline. 3. Sodium hypochloride(1:40 dilution) in sterile saline. 4. Saline solution (400 cc) with 500000 units of

benzyl penicillin and 1 gram of streptomycin sulphate. 5. Saline solution (400 cc) with 1 gram kanamycin sulphate. The preserved grafts were kept in refrigerator at 4°C of temperature. He studied the preserved membrane bacteriologically at regular intervals i.e. 1st, 3rd, 7th, 14th and 30th days to test the efficiency of different solutions. He used the grafts upto one month of preservation. He concluded solution 3rd, 4th and 5th to be best. He showed cellular necrosis started from 2nd day. However according to Kirschbain and Hernaudaz (1963) cellular elements survived even after 45-50 days.

Trelfored and associates (1972) reported preliminary results after using amnion alone as autograft and allograft from sheep. They also observed the mesenchymal side application towards the host, to be more consistent 'take'.

Martin (1972) compared the phagocytic activity of different bio-dressings in an experimental study. He produced 20% full thickness scald on back of 50 Aprague dwley rats and 10^8 pseudomonas aerugenosa were inoculated in each wound. After 5 days eschar tissue from each of surviving 38 animals were biopsied for quantitative and qualitative bacteriological studies. Then the wounds were dressed with human skin or amniotic membrane while some were left open as control cases. The

dressings were changed after every 48 hours. The wounds biopsied and bacteriological count were performed after 96 hours or after two changes in the biological dressing. The bacterial count was markedly descreased in all the animals treated with biodressings. Only 40% of control cases showed decrease in bacterial count. The degree of decrease was a thousand time greater by amniotic membrane than skin.

In vitro, he denied the presence of any specific antibacterial agent in amniotic membrane homografts. In vivo, the antibacterial effect is due to host's own defence mechanisms in biologically closed wounds obtained with bio-dressing.

Trelforêd and associates (1975) reported the use of amnion alone in full thickness fresh surgical wounds. They also suggested that the mesenchymal surface provides better 'take'. He observed decrease in pain, fluid loss and secondary infection, and hence decrease in hospital stay.

In further studies Martin (1973) showed clear inhibitory effect of amniotic membrane on microbial growth. He also observed the autolysis and further disintegration of amniotic membrane in 48 hours if applied over deep grossly infected burn wound.

Gullermo Colocho et al (1974) in their clinical and experimental studies applied amniotic membrane over open wounds (60 patients with full thickness donor sites and 42 patients with partial thickness burn) and in subcutaneous pockets in rats. He observed that amnion dessicated over partial thickness wound and reepithelization of skin began on 3rd day and completed on 7th day. There was no evidence of allergy, rejection or neovascularization in any case. In the experimental study amnion retained it's cellular integrity when buried beneath the flaps even after 5 days. None of the human donor site biopsy and India ink injection in experimental animal showed communication with the host.

C.V. Bapat and Promod M Kothari (1974) reported the successful restoration of floor of mouth with human amniotic membrane after radical total glossectomy.

Martin further observed in 1976 the use of amniotic membrane as most rapid way of reducing bacterial population in operative wounds. The relief in pain was similar to that after use of allegraft skin. Amniotic membrane adhere better than xenograft.

Marilyn Trelford - Sauder and others (1977) used amniotic membrane to cover raw area after

pelvic exentration. They concluded following benefits- It is readily available tissue of low antigenecity, low intestinal complications because of absence of pelvic raw surface, reduced protein and fluid loss and reduced hospital stay. In 1978 they reported the use of allograft amniotid membrane to prevent intra abdominal adhesions.

Bose B (Nov. 79) recently reported the use of amniotic membrane over burn wound especially in developing country like ours.

COLLAGEN SHEET

Collagen is a connective tissue which is present in many of the animal tissues like skin, muscle, bone etc, in high concentration. It is well established that collagen in its pure form has minimal antigenic activity. This property of collagen makes it an ideally suited raw material for use in the preperation of a varéety of biomaterials for use in surgery.

For more than a century collagen rich mammalian intestine are being used for the manufacture of absorbable surgical sutures. During the last 40 years another surgical productm made out of gelatin (hydrolysed collagen) in the form of foam is also being successfully used by surgeons for arresting hemorrhage. In recent times there had been attempts at utilizing collagen for making gels, powders, tapes, sheets etc, for use in various surgical situations.

Collagen sheet and it's processing -

Collagen sheet is a product made from animal tissues rich in collagen such as serosa and submucous layers of caecum and/or intestines, pericardium, amniotic membrane etc. This raw material is collected from sloughtered healthy animals. It is thoroughly cleaned by washing it repeatdly in warm water and chemically freed from the undesirable non-collagenous proteins, lipids and mucopolysaccharides etc, with successive treatment in baths of non-ionic detergent alkali and oxidising agents. To enhance the in-vivo time of digestion, the alkali free stock is crosslinked in a solution of a suitable cross linking agent such as chromium sulphate. The chromicised stock is then washed well to make it free from soluble and processed into sheets of desired size. The sheets so produced are packed either in glass ampoules or polythylene sackets with a preserving fluid containing ethylene oxide as sterilizing agent and hermetically sealed.

Clinical applications-

Pandey et al successfully used collagen sheet in the cases of excision orthroplasty, in prevention of ankylosis. Thukral used it in repair of hernia and other surgical defects. Gupta et al used it as coverage material in artificially produced raw area in

an experimental study. Bhargava et al showed it's usefullness in brigding abdominal wall defects. Kumar also used it to gap the abdominal defects. Ibrahim showed the use of collagen sheet in vestibuloplasty.

MATERIAL AND METHODS

MATERIAL AND METHOD

The present study has been conducted at M.L.B. Medical College Hospital, Jhansi from May 1981 to April 1982 to compare the effects of amniotic membrane and collagen sheet application over superficial thermal burns as bio-dressings.

Collection of Amniotic membrane

The amniotic membranes were collected from the labour rooms and obstetric operation theatres of M.L.B. Medical College Hospital and District Hospital Jhansi at the time of labour or caesarian section. The mothers having intact membranes and without any history suggestive of genital tract infections, were selected for collection of amniotic membrane. Parity and blood group of mother were not considered. The placenta with intact membrane was taken directly in a clean tray and was washed thoroughly in running tap water to remove blood and mucoid material.

Seperation of Amniotic membrane from the placenta

The thoroughly washed placenta was transferred to another clean tray filled with water. The amniotic membrane was seperated from chorion and placenta, gently starting from the periphery up to the base of the umblical cord. The seperated amniotic membrane was cut at the base of umblical cord and spread over flat

surface in a sterile container filled with sterile normal saline. The remaining clots were removed gently from its surface with the help of sterlized gauze pieces. It was further cleaned with sterile normal saline. The amniotic membrane thus obtained was thin, transparent, elastic and shining with whitish hue.

Preservation of amniotic membrane

Amniotic membranes were preserved in the sterile infusion bottles of normal saline treated with 10 lac units of Benzyl penicillin and 1 gram of streptomycin sulphate. These bottles were kept in refrigerator at 4°C till the time of application. The preserved membrane was continuously watched for bad odour and change in colour from white to yellow or brown.

Collagen sheet

The collagen sheets were supplied by Central Leather Research Institute Adyar, Madras in glass ampoules with a preserving fluid containing ethelene oxide.

Selection of cases

All the cases with superficial burns of less than 50% of body surface who came to the emergency or out patient department of this hospital with in 72 hours of the thermal injury were included in this study irrespective of their age, sex, socioeconomic status, contamination of wound and mode of injury.

Method of study

History and physical examination

The selected cases were subjected to detailed history and physical examination which were recorded on following lines -

History

Introduction - Name, Age, Sex, Occupation, Rural/ Urban, Address, Date of admission, Date of discharge and Time of healing.

Regarding the burn accident

- Date and time of burn (duration of burn).
- Place of accident and nature of work at the time of accident.
- Cause of burn.
- Prior treatment (if any).
- Symptoms.

Physical examination

General Examination - The case was examinedfor general condition, pulse, blood pressure, temperature, respiration and hydration.

Local Examination -

(A) Percentage of burn - It was calculated by ' Wallace's rule of Nine ' in the adult and by " Lund and Browder Chart " in children.

(B) Depth of burn - Superficial/Deep.

Estimation of depth of burn

The hypodermic needle was used to test the pain sensation. The area with increased sensibility wasconsidered to be superficial or partial thickness burn. The area with markedly reduced or absent pain sensibility was considered to be deep or full thickness burn. This was also confirmed by pulling out a hair from burn surface. In the 3rd degree or deep burn, hair pulls out easily and painlessly. The later test is of value in borderline cases of second degree burn. In addition, help of the following criteria was also sought.

Classification of depth	Appearance of burn area	Pain sensation
Ist degree	Erythematous	Painful and hyper-aesthetic.
IInd degree		
(A)	Blisters with reddened base and moisture	Painful and hyper-aesthetic.
(B)	Blisters with blanched base and moisture	Painful, hyper-aesthetic or anaesthetic at places.
IIIrd degree	Leathery pale or pearly white or charred dry	Painless and anaesthetic.

The I and II(A) were included as superficial and II(B) and III were considered as deep burn.

(C) Contamination of wound

Apparently clean: No contamination of foreign body, clean intact blisters.

Mild Contamination: Slight contamination, ruptured blisters, open wounds.

Gross contamination: Heavy contamination with dirty cloth, foreign body, dust, and/or non medical substances i.e. cowdung, mud etc.

(D) Area involved - Diagrammatic representation in anterior posterior and lateral views (shown in attached proforma) was done.

Resuscitation and general treatment

The resuscitation was done and treatment was started on conventional lines (i.e. I/V infusions, blood and plasma transfusion, analgesic, antibiotics and tetanus prophylaxis).

Local management of wound

On the basis of local management of wound patients were divided in to following groups:-

Group A - Amniotic membrane was applied over full burn area.

Group B - Collagen sheet was applied over full burn area.

Group C - Amniotic membrane was applied over a part of burn area (C_1) and remaining area was treated by open gauze piece application with Betadine or Silver Sulfadiazine (C_2).

Group D - Collagen sheets were applied over a part of burn area (D_1) and remaining area was treated

by open gauze piece application with Betadine or Silver Sulfadiazine (D_2).

Group E - Amniotic membrane was applied over one part of burn area and collagen sheet on other part.

Preparation of burn surface

Swab from burn surface was taken for culture and senstivity test. The patient was given necessary sedation. A gentle but thorough debridement of wound was done by removing necrosed skin and blisters. The area was again tested for degree of burn. Then the wound was cleaned with 0.5% savlon solution twice followed by sterile normal saline thoroughly. The spirit was applied over the adjacent skin around the margin of wound area. Now the wound area was dressed locally according to the proposed group.

Application of amniotic membrane:

Fresh or preserved amniotic membrane was taken out from the bottle with the help of forceps. The membrane with bad odour and colour changes were discarded. It was stretched open and then applied over burn area about one inch beyond the margins. The temperature of membrane was not considered. The air bubbles between membrane and wound area were removed. The patients were instructed not to move the part until the membrane became adhered and relatively dry. It was left as such without any dressing except in children and uncooperative

patients where the dressing was applied to retain the membrane.

Application of collagen sheets

The collagen sheet was taken out from glass ampoules by cutting it at the marker in the middle of it. It was washed in sterile normal saline (obtained from sealed bottle) to remove it's preservative ethylene oxide which is very much irritant to raw burn area. The sheet was spread over and made to dry for half a minute. Thenit was applied over burn area covering atleast 1-2 cm of adjoining normal skin around burn margins. The air bubbles were removed. Dressing were applied in all the cases to retain it in place and removed after 12 hours. If the sheet adhered firmly after 12 hours no further dressings were applied. If not then dressings were applied again for further 12 hours.

Application of antibiotic gauze pieces

The gauze pieces soaked in antibacterial agents were applied over raw surface to cover it fully. Betadine, Silversulfadizine solution and cream, Soframycin and Gentacyn cream were used as local antimicrobial agents. These dressings were changed regularly as indicated by soakage and discharge.

Assessment of the case

The assessment of the results was done

by interview with patient, examination at regular visits and investigations.

Interview - The patient was asked about:

1. Pain and discomfort (Mild, Moderate or Severe)
2. Fever
3. Any evidence of allergy as itching, rashes, nausea and vomiting.

Physical examination

General examination - The case was examined for general condition, hydration, pulse, blood pressure and signs of toxaemia.

Local examination - Observation for the following was done -

1. Presence of discharge and/or soakage.
2. Appearance of amniotic membrane and/or collagen sheet as regard to surface, margin, thickness, lusture, colour, dryness and adherence.
3. Appearance of burn area covered with antibiotic gauze pieces.
4. Collection of pus under dressing - If the pus was localized in small area underneath amniotic and/or collagen sheet,a slit was given in it. A pus swab was taken for culture and sensitivity test. If the pus is underneath whole of membrane and/or collagen sheet or

localized at many places, then the biological dressing was removed and burn surface was treated with antibiotic gauze pieces.

(5). Result of healing.

Investigations

1. Routine : Blood-complete haemogram.

Urine-gross and microscopic examination.

2. Culture and sensitivity test for pus if present:

This was cultured on blood agar and chocolate agar media, which were kept in refrigerator at 0-4°C temperature for 24 hours. Antibiotic sensitivity was done in the cases, where growth of pathogenic bacteria was revealed.

PROFORMA

Name	**Age/Sex**
Occupation	**Rural/Urban**
Address	**Date and time of admission.**
	Date and time of discharge.
Group	Total time of healing

HISTORY

(i) Date and time of burn

(ii) Place of work and nature of work at the time of burn

(iii) Cause of burn

(iv) Prior treatment (if any)

SYMPTOMS

(i) Pain

(ii) Burning

(iii) Blisters

(iv) Fever

(v) Oliguria

(vi) Discharge from wound surface

(vii) Difficulty in swallowing or in inspiration

(viii) Any other

PHYSICAL EXAMINATION

(a) General examination at time of admission

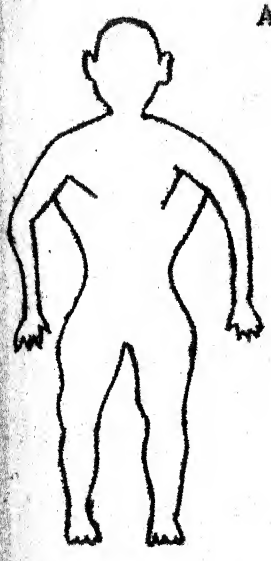
-G.C. -Pulse -B.P.

-Temperature -Hydration

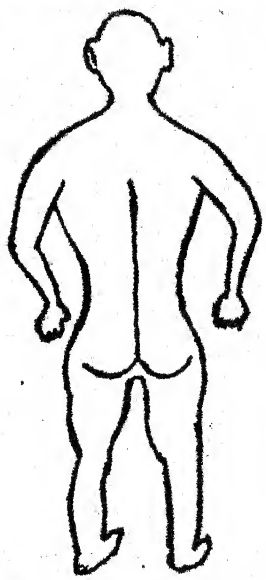
(b) Local examination

- Percentage of burn
- Depth of burn/Degree of burn
- Contamination
- Appearance of raw area
- Area involved (Diagramatic)

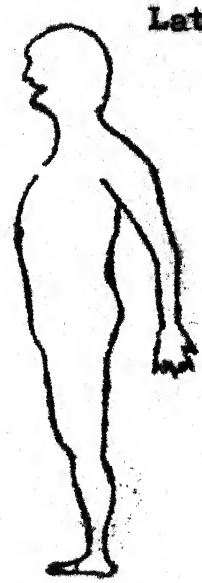
Anterior



Posterior



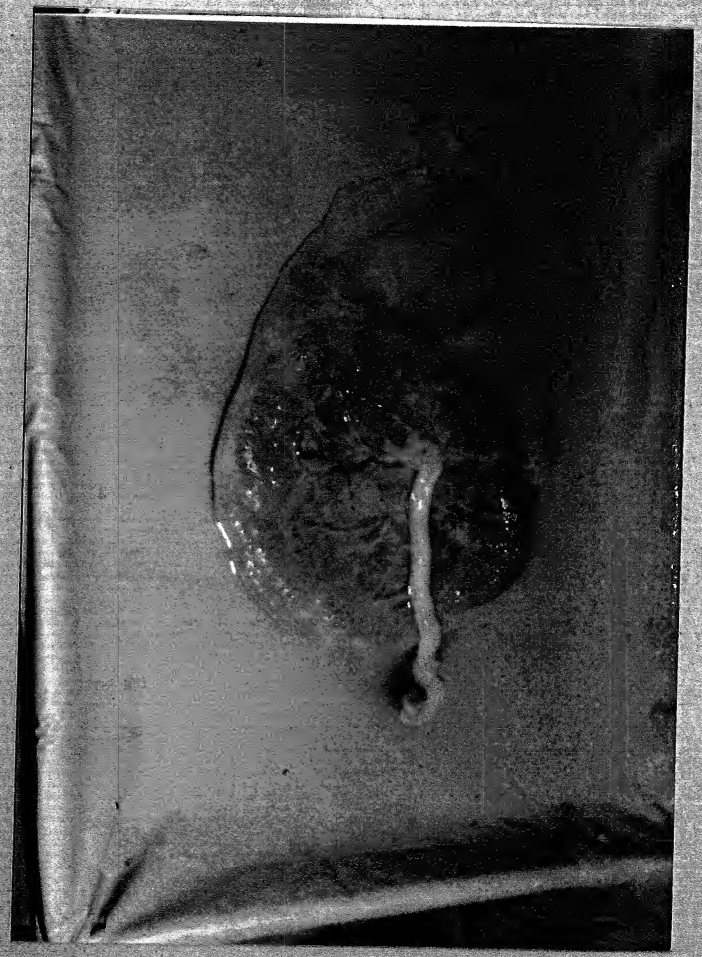
Lateral



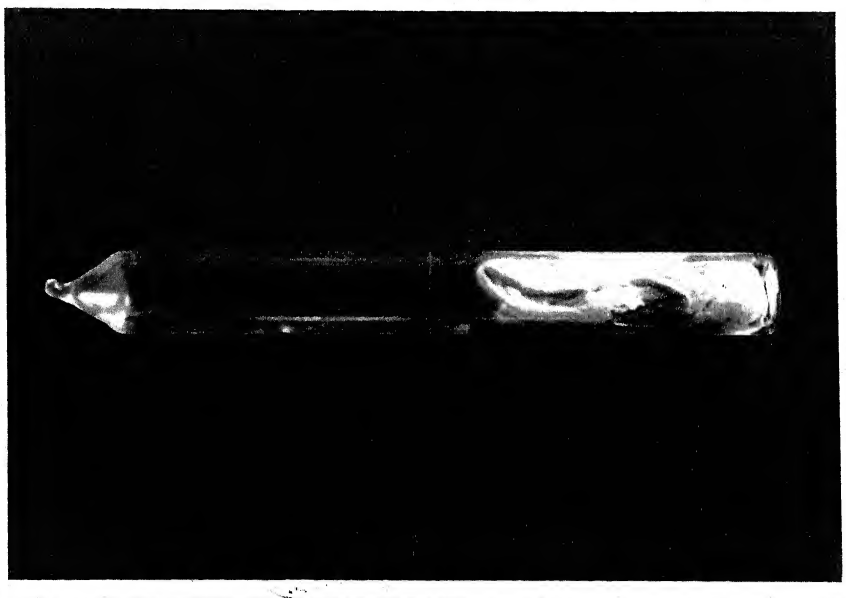
Progressive report -

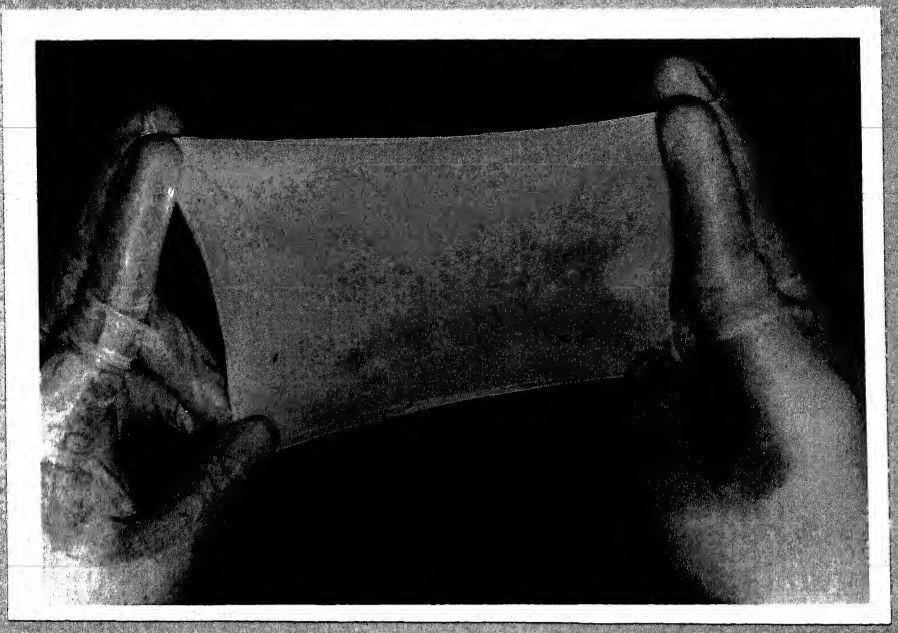
Days	1	2	3	4	5	7	9	12	14	18	21	25	30	35
G.C.														
Pulse														
B.P.														
Hydration														
Temperature														
Difference between input & output														
Albumin in urine														

	Wound dressed with amniotic membrane													Wound dressed with collagen sheet										
Days	1	2	3	4	5	7	9	12	15	18	22	25	30	1	2	3	4	5	7	9	12	15	18	22
Pain																								
Soakage																								
Mobility																								
Biodressing changes-																								
(a)Surface																								
(b)Margins																								
(c)Thickness																								
(d)Lusture																								
(e)Colour																								
(f)Dryness																								
(g)Adherence																								
Healed on																								
Time in healing																								




SODIUM CHLORIDE INJECTION
I.P. 0.9 W.V.





OBSERVATIONS

O B S E R V A T I O N S

This study comprises of 41 patients of superficial burn admitted in indoor, emergency wards or who attended surgery out-patient department at M.L.B. Medical College Hospital, Jhansi from May 1981 to April 82. These patients came to hospital with in 72 hours of burn accidents. These patients were of different age, sex and occupations. All the patients included had burns involving less than 50% of body area.

Out of 41 cases studied 22 were female and 19 were male. Incidence of the burn injury was same in urban and rural population. Out of 41 cases, 21 were rural and 20 were urban inhabitants. 36 out of them were indoor patients and 5 of them were treated as O.P.D. patients.

Table No. 1

Showing age incidence:-

S.No.	Age group (years)	Number of cases	%
1.	0-10	10	24.3
2.	11-20	15	36.6
3.	21-30	12	29.3
4.	31-40	3	7.3
5.	41-50	1	2.5
6.	51-60	0	-
Total		41	100

Table No. 2

Showing sex distribution in different age groups:-

S.No.	Age group	Male		Female		Total	
		No.	%	No.	%	No.	%
1.	0-10	6	60	4	40	10	24.3
2.	11-20	7	46.6	8	53.4	15	36.6
3.	21-30	4	33.3	8	66.7	12	29.3
4.	31-40	1	33.3	2	66.7	3	7.3
5.	41-50	1	100	-	-	1	2.5
Total		19	46.3	22	53.7	41	100

Most of the burn cases were in their eakly age groups. 37 patients out of 41 were below the age of 30 years. About half of the cases were between the age of 15-25 years i.e. 20 cases out of 41 cases. 15 cases (36.6%) were between the age of 18-23 years. The oldest case in this study was a 42 years old male and youngest was a 2 years old male child. It was observed that males were 1½ times more sufferer than females between the age of 1-10 years while on the contrast females were 1½ times more sufferer between the age of 11-30 years.

Table No. 3

Showing location of burn accident according to sex -

S.No.	Location	Male		Female		Total	
		No.	%	No.	%	No.	%
1.	Indoor	8	28.5	20	71.5	28	68.3
2.	Outdoor	11	84.7	2	15.3	13	31.7
Total		19	46.3	22	53.6	41	100

It was observed that the most of the burn accidents (68.2%) occured at home out of these victims 71.4% were females while in the outdoor accidents 84% victims were male.

Table No. 4

Showing the cause of burn according to sex -

S.No.	Cause of burn	Male	Female	Total	
				No.	%
1.	Fire during cooking	6	12	23	56.1
2.	Kerosene lamp	2	10	2	4.9
3.	Kerosene stove	0	2	2	4.9
4.	Petrol/Diesel	3	0	3	7.3
5.	Scalds	2	3	5	12.2
6.	Electric	2	0	2	4.9
7.	Hot metallic rod	0	1	1	2.4
8.	Miscelleneous	1	2	3	7.3
Total		19	22	41	100

It was observed that fire during cooking was the main causative factor. 23 (56%) cases out of 41 were victims of this fire and out of them 17 were females. Interestingly 4 children were burnt while swimming in a pond and the diesel leaked from pumping engine got fire. In the miscelleneous group the patient sustained burn due to crakkers and physical disability as due to epilepsy.

31% were having mildly contaminated wounds. The contamination was directly propotional to time gap between the accident and hospital admission. Only 2 cases were grossly contaminated, one of them by plant leaves and other by cowdung.

Table No. 7

Showing the number of cases in different groups according to local management of wound-

S.No.	Group	Number of cases	%
1.	A	12	29.3
2.	B	9	21.0
3.	C	3	7.3
4.	D	3	7.3
5.	E	14	34.1
Total		41	100

Wound surfaces involving similar percentage of body areas were kept for 2 different types of bio-dressings (as shown in table No.8).

Occupationwise 39% were house-wives and 12.1% were students among the patients. It is observed that persons from all walks of life were included in this study. It is also observed that maximum number of patients (24.3%) came in the month of October and minimum in the month of March and April.

Table No. 8

Showing the percentage of burn surface over body treated by two different bio-dressings -

S. No.	% of burn	Amniotic membrane application			Total	Collagen sheet application			Total	Antibiotic gauze piece application		Total
		A	C_1	E_1		B	D_1	E_2		C_2	D_2	
1.	0-10	2	1	7	10	5	1	5	11	1	1	2
2.	11-20	5	2	5	12	2	-	6	8	2	1	3
3.	21-30	3	-	2	5	2	1	3	6	-	1	1
4.	31-40	1	-	-	1	-	1	-	1	-	-	-
5.	41-50	1	-	-	1	-	-	-	-	-	-	-
Total		12	3	14	29	9	3	14	26	3	3	6

Table No. 9

Showing number of cases in different months -

S.No.	Month	Number of cases	%
1.	January	5	12.2
2.	February	3	7.3
3.	March	0	0
4.	April	1	2.5
5.	May	4	9.7
6.	June	4	9.7
7.	July	2	4.9
8.	August	2	4.9
9.	September	2	4.9
10.	October	10	24.5
11.	November	4	9.7
12.	December	4	9.7
Total		41	100

Amniotic membrane applied was either freshly obtain or preserved. The freshly obtain membrane was treated first with normal saline having 10 lac units of crystalline penicillin and 1 gram steptomycin sulphate. One amniotic membrane preserved even upto 18 days, was used without any ill effect. During preservation it was observed that amniotic membrane became thick and slightly opalascent. The membrane was preserved upto one month and then it was not used and discarded.

Table No. 12

Showing different age groups in burn in different groups according to local management -

S. No.	Age group	A(%)	B(%)	C(%)	D(%)	E(%)	Total
1.	0-10	2(16.6)	5(55.6)	-	-	3(21.4)	10
2.	11-20	7(58.4)	2(22.2)	2(66.7)	1(33.3)	3(21.4)	15
3.	21-30	3(25)	2(22.2)	1(33.3)	2(66.6)	4(28.6)	12
4.	31-40	-	-	-	-	3(21.4)	3
5.	41-50	-	-	-	-	1(7.2)	1
Total		12	9	3	3	14	41

Immediately after the application of bio-dressing, the 26 wound surfaces covered with amniotic membrane (A, C_1, E_1) were relieved of pain and discomfort out of 29 while the pain persisted in 24 out of 26 wound surfaces covered with collagen sheet (B, D_1, E_2). The 3 wounds areas covered with amniotic membrane in which

pain persisted were of group A. One of them rejected the amniotic membrane and two of them got infection underneath which died afterwards. All of them were having more than 30% burn. While pain persisted in 92.1% wound areas covered with collagen sheet, none of them rejected the sheet. There was no symptom suggestive of allergic reaction in any case dressed with 2 different bio-dressings.

Table No. 13

Showing the immediate effects of different types of dressings -

S. No.	Type of dressing	Group	Total wound areas	Pain relieved	Pain Persisted	Allergic reactions Rigors	Rashes	Vomiting
I.	Amniotic membrane application	A	12	9	3	-	-	-
		C_1	3	6	6	-	-	-
		E_1	14	14	-	-	-	-
Total			29	26	3	-	-	-
II.	Collagen sheet application	B	9	0	9	-	-	-
		D_1	3	0	3	-	-	-
		E_2	14	2	12	-	-	-
Total			26	2	24	-	-	-
III.	Antibiotic gauze piece dressing	C_2	3	0	3	-	-	-
		D_2	3	0	3	-	-	-
Total			6	0	6	-	-	-
Total of I+II+III			60	28	33	-	-	-

Table No. 14

Showing the number of cases in collagen sheet applied cases in which pain relieved after different duration-

S.No.	Time interval after which pain relieved	B	D_1	E_2	Total wound areas	
					No.	%
1.	0-6	6	2	9	17	70.8
2.	7-12	2	1	2	5	20.8
3.	12-18	1	-	1	2	8.4
Total		9	3	12	24	100

In collagen sheet covered 24 wound areas, pain was relieved in 70.8% wounds with in 6 hours and in 20.8% within 12 hours. No pain was observed in any case after 18 hours. Only in 2 wound areas pain persisted for 12-18 hours. In fact in 8 areas (33%) pain increased and burning appeared just after application of collagen shhet.

Slight discomfort or feeling of slight stretching was observed when the bio-dressings became adhered to raw surface. Slight itching in all the healed areas was observed 1-2 days prior to peeling off of the bio-dressing.

As regards the changes in bio-dressings it was observed that surface of both the bio-dressing remained smooth upto 24-36 hours after application.

Collagen sheet was relatively rough surfaced. After 24-36 hours surface was wrinkled in both the cases. The wrinkles in amniotic membrane were irregular in size amplitude and direction while in collagen sheet wrinkles were either in transverse or longitudinal direction. During wrinkling both the bio-dressings remained firmly adhered to underlying wound surface.

In 22 wound surfaces out of 26 amniotic membrane was 'taken up' the membranen adhered to raw area between 6-12 hours, only 4 cases took 12-18 hours for amniotic membrane to be adhered to raw surface. In all the collagen sheet applied areas the sheet adhered completely to raw surface between 12-18 hours. The amniotic membrane became dry in 6-12 hours in 75.9% wound surfaces while the collagen sheet became dry between 4-16 hours after application in all the cases.

For the margins of amniotic membrane started curling up, it was observed, from adjacent normal skin within 6-20 hours and from the margins of wound after 9-15 days. The margins of collagen sheet curled from normal skin after 4-8 hours and from margin of wound after 7-14 days.

The thickness appeared to be increased of both the bio-dressings when they became dry and

adhered. The exact increase in thickness of bio-dressing could not be made out. The lusture of amniotic membrane was lost in 6-18 hours after application. While the collagen sheets were lustureless from the beginning.

It was observed that colour changes were slower in amniotic membrane than that in collagen sheet. The amniotic membrane became hazy after 6-16 hours and its hazyness increased till it became opaque after 36-52 hours after application. Then it's colour changed to brownish after 6-9 days. The collagen sheet was hazy when taken out from glass ampoules and applied over raw surface. Then it became transparent after 12-28 hours. It turns to be opaque after 5-9 days with whitish fibres becoming prominent in longitudinal direction.

It was observed that in all the non-rejected cases of amniotic membrane and collagen sheet applied wounds, patient was able to move his part and was well subjected to active and passive physiotherapy as soon as the bio-dressings became adhered and dry. This achieved mobility was a contributory factor in the prevention of contractures. In amniotic membrane applied cases it was achieved slight earlier because of it's earlier adherence and drying. It took 4-16 hours in amniotic membrane applied areas to move the part.

Table No. 15

Showing the incidence of soakage and pus formation and microorganism in different groups-

S. No.	Type of dressing	Groups	Total number of burn area	Soakage	Pus	Causative microorganism
1.	Amniotic membrane application	A	12	0	3	Pseudomonas aeruginosa, Staphylococus aureus
2.		D_1	3	1	-	Sterile
3.		E_1	14	0	1	Staphylococus aureus
Total			29	1	4	
4.	Collagen sheet application	B	9	1	0	Sterile
5.		D_1	3	1	1	E.Coli
6.		E_2	14	1	0	Sterile
Total			26	3	1	
7.	Antibiotic gauze pieces application	C_2	3	3	3	Staphylococus aureus, E.Coli, Pseudomonas aeruginosa.
8.		D_2	3	3	3	E.Coli, Staphylococus aureus
Total			6	6	6	

It has been observed that 5 out of 29 amniotic membrane covered wound areas,had soakage and 4 out of them showed pus formation clinically. All these revealed growth of microorganism, in one case pseudomonas, and three cases staphylocococi aureus after culture and sensitivity test. One case did not revealed growth of any microorganism. 3 out of 5 infected surfaces were of group A and of more than 30% of burn surface.

On the other hand 3 out of 26 collagen sheet covered areas, had soakage and one of them showed pus formation clinically. It revealed the growth of E.Coli. All the wound areas treated with anti-biotic gauze pieces got infection and the microorganisms were staphylococus aureus, E.Coli and pseudomonas.

It was observed that 22 wound areas (75.9%), treated with amniotic membrane showed complete healing while 4 (13.7%) showed partial healing. The partial healing denotes when whole of the area was not healed after peeling off membrane and there were small areas of healthy granulation tissue. There was rejection in 3 cases only. The rejection in all the 3 cases was due to formation of pus pockets beneath the membrane. Two out of them died later.

In the collagen sheet applied cases, there was no mortality and no rejection. 24 out of 26

Table No. 16

Showing the results in each group -

S. No.	Type of dressing	Group	Total wound areas	Rejection	Mortality	Skin grafting required	Total healing	Partial healing	Contracture
1.	Amniotic membrane application	A	12	2	2	0	8	2	0
2.		C_1	3	0	0	1	2	1	0
3.		E_1	14	1	0	0	12	1	0
Total			29	3	2	1	22	4	
4.	Collagen sheet application	B	9	0	0	0	8	1	0
5.		D_1	3	0	0	0	3	0	0
6.		B_2	14	0	0	0	13	1	0
Total			26	0	0	0	24	2	0
7.	Antibiotic gauze piece application	C_2	3	0	0	2	0	0	1
8.		D_2	3	0	0	1	0	0	1
Total			6	0	0	3	0	0	2

wound areas showed complete healing i.e. 92.3% and only two (7.6%) showed partial healing. In none of the areas skingrafting was required where bio-dressings were applied. None of the bio-dressing covered area showed any contracture. While out of 6 antibiotics gauze piece applied areas skin grafting was done in 3 areas, and contracture also developed in 2 areas even after vigorous physiotherapy.

Table No. 17

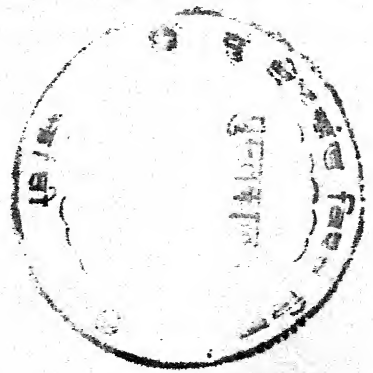
Showing the time taken in total healing in each group of cases -

S. No.	Time taken in total healing (days)	Amniotic membrane application			Collagen sheet application			Antibiotic gauze piece application	
		A	C_1	E_1	B	D_1	E_2	C_2	D_2
1.	6-10	-	-	-	-	-	-	-	-
2.	11-15	1	-	2	6	-	8	-	-
3.	16-20	7	2	8	2	2	5	-	-
4.	21-25	1	1	3	-	1	-	-	-
5.	26-30	-	-	-	1	-	1	1	1
6.	31-35	1	-	1	-	-	-	-	2
7.	36-40	-	-	-	-	-	-	-	-
8.	41-45	-	-	-	-	-	-	1	-
9.	46-50	-	-	-	-	-	-	-	-
10.	51-55	-	-	-	-	-	-	-	-
11.	56-60	-	-	-	-	-	-	1	-
12.	60 onwords	-	-	-	-	-	-	-	-
Total		10	3	14	9	3	14	3	3

Table No. 18

Showing the total time of healing in different dressings in different percentage of burn surface -

S. No.	Time taken in total healing (days)	Amniotic membrane treated wound areas(29) Percentage of burn area					Collagen sheet treated wound areas(26) Percentage of burn area				
		0-10	11-20	21-30	31-40	41-50	0-10	11-20	21-30	31-40	41-50
1.	6-10	-	-	-	-	-	-	-	-	-	-
2.	11-15	3	-	-	-	-	11	3	-	-	-
3.	16-20	6	9	2	-	-	-	5	4	-	-
4.	21-25	1	3	1	-	-	-	-	1	-	-
5.	26-30	-	-	-	-	-	-	-	1	1	-
6.	31-35	-	-	1	1	-	-	-	-	-	-
7.	36 on words	-	-	-	-	-	-	-	-	-	-



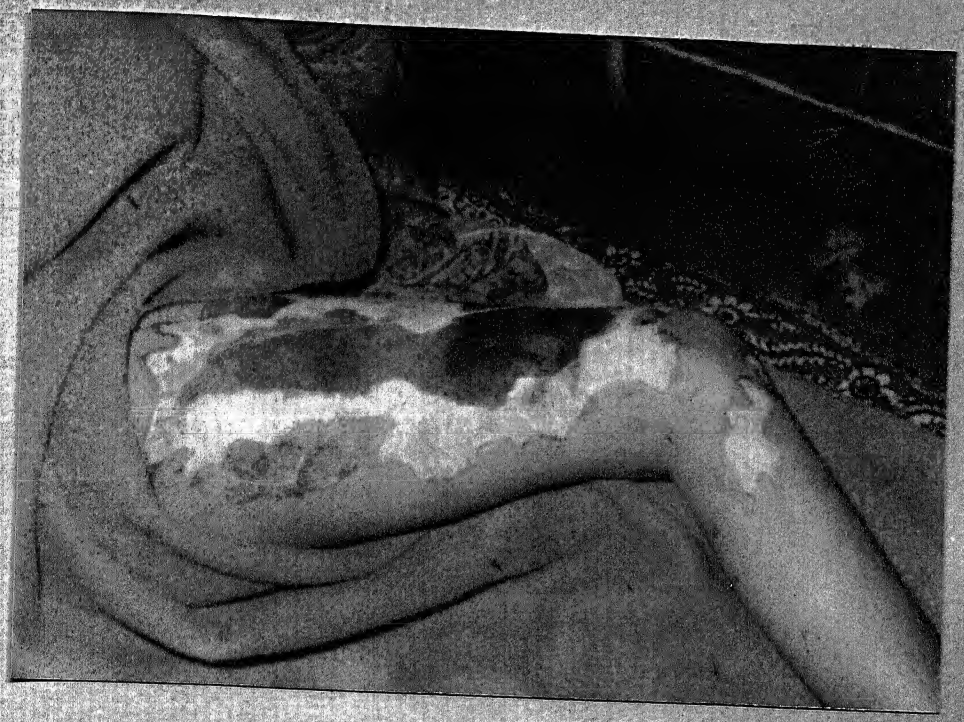
It is observed that in 'takeup' cases amniotic membrane peeled off itself after 14-24 days leaving complete healed wound area or small areas of healthy granulation tissue i.e. partial healed areas. Out of 26 'takepu' areas 3 areas healed within 11-15 days, 17(65.3%) healed within 15-20 days and 2(7.6%) within 25 days. Out of 4 cases where partial healing took place 2 of them healed within 20-25 days and 2 with in 30-35 days.

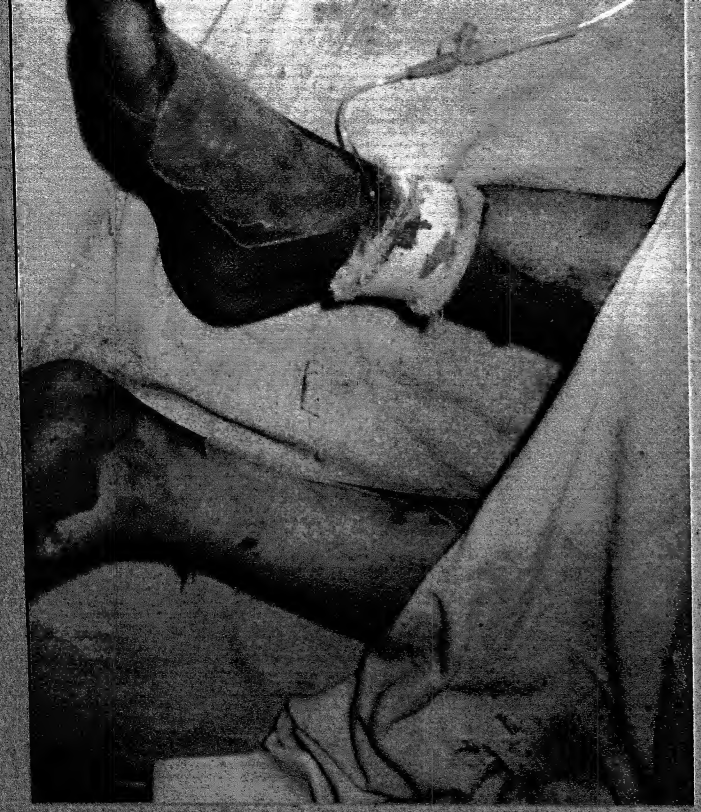
Out of 26 collagen sheet applied areas 14 healed completely within 11-15 days, 9 within 15-20 days, 1 within 22 days. Two cases took 22-30 days for total healing. These two were the cases where partial healing was observed after peeling off of collagen sheet. Out of the six areas treated with antibiotic gauze pieces all healed after 25 days. Two of them healed within 26-30 days two within 31-35 days and remaining 2 after 40 days.

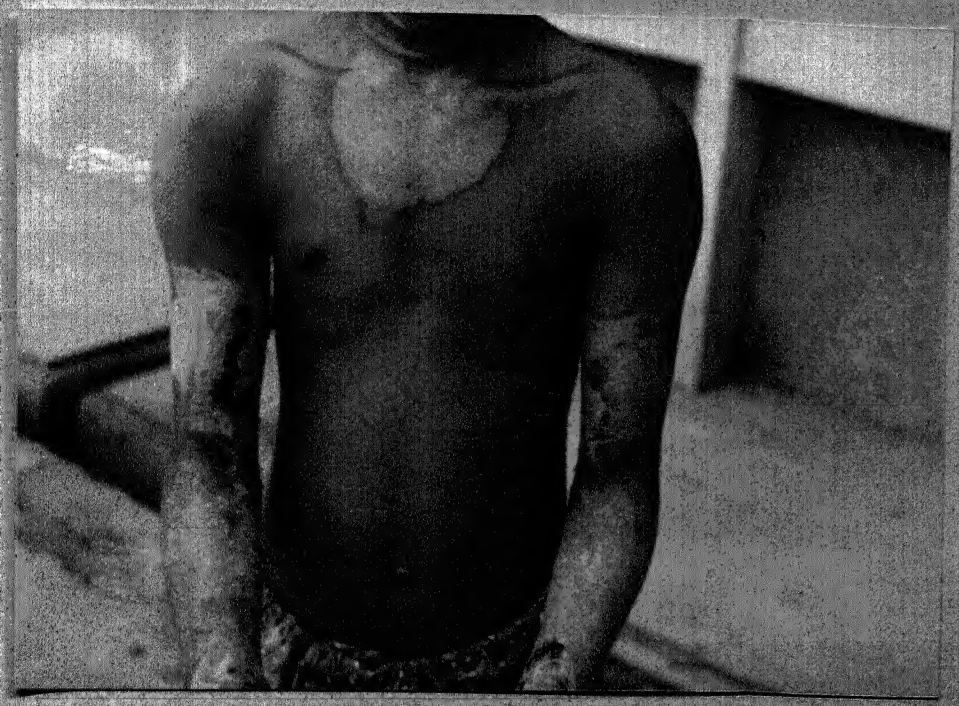
To compare the time taken in total healing of areas covered with two different bio-dressings in similar percentage of burn area, it was observed that out of 10 amniotic membrane applied areas below 10% burn, three healed completely within 11-15 days, six within 16-20 days and one within 21-25 days. While all the 11 collagen sheet applied areas of similar burn percentage

healed within 11-15 days. Out of 12 amniotic membrane applied areas of 11-20 percent burn, nine healed completely within 16-20 days and 3 within 21-25 days, while out of 8 collagen sheet applied areas of similar percentage of burn 3 healed completely within 11-15 days and 5 within 16-20 days. Noneof them took more than 20 days. Out of 4 amniotic membrane applied areas of 21-30% burn two healed completely within 15-20 days, 1 in 23 days and remaining in 32 days. But when collagen sheet was applied over six areas of similar percentage of burn four of them healed within 15-20 days, 1 after 22 days and one after 26 days. In the areas of 31-40% burn, 1 dressed with amniotic membrane healed after 32 days and other dressed with collagen sheet healed after 26 days.

The scar after healing in amniotic membrane treated areas were pink, smooth with flat margins in 22 areas (75.6%) out of 26 'takeup' areas. The remaining 4 areas healed with red scar having raised surface and margin. The scar after healing in collagen sheet applied areas were pink with smooth and flat margin in 24 out of total 26 areas.







DISCUSSION

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Burns are notorious in the sense that they break continuity of skin and produce a raw area. The water retention ability of skin depends on its effective vapour pressure and diffusion barrier offered by keratin layer and lipid contents in the stratum corneum. This lipid is thermolabile. When this barrier is removed after thermal injuries, the effective vapour pressure gradient is increased by 15-20 times. This results into a larger amount of evaporative water loss amounting the increase to 3-10 times. The amount and duration to which the loss persits depends on the depth and percentage of burn.

The loss of blood flow which occurs initially, starts returning towards normal after 24 hours as patent vessels reappear. The process of revascularization is associated with local circulatory stasis. This stasis makes the wound vulnerable to dessication and infection. Either of these can trip the balance converting it to a zone of necrosis or full thickness burn. The infection is the main factor for mortality and morbidity during hospital stay and afterwards in the form of discolouration, scar formation and contractures.

Therefore the main emphasis, in the mana-

epithelial cells preserved for reepithelization, is on covering the raw area and thus making it a close wound which subsequently reduces excessive evaporative water loss and prevents wound infection.

The autografts are best to cover the raw area but these have limitations particularly when the area is large, patient is not fit for surgery and the patient or relatives do not consent for it, on religious, sentimental or ethical grounds. Alternatively homografts are used but here again availability is limited. Other biological covering materials are allograft skin, hetrograft skin, collagen sheet, foetal membranes. The cadaver skin is in limited supply in general hospitals. Secondly they are very expensive and thus not feasable for a poor country like ours. Baxter has estimated that six physician hours and hospital cost of $ 225 are needed per patient for cadaver skin grafting.

The present work is a study of effects of two bio-dressing coverage over superficial burns and to assess the superiority of either of them. This study was done over the patients of superficial burns of less than 50% of body area involvement without considering the factors of age, sex, occupation, socio-economic status inhabitancy, mode and cause of burn and contamination of wound.

As regards the incidence of burn no

significant difference was observed in sex and inhabitancy. Significantly it was observed that females were more sufferer tha males in the age group of 15-30 years. In our country the females of this age group are housewives in middle class families. They are indulged maximally in house hold work and thus are more liable to thermal injury by fire appliances. It is in accordance with other studies published from time to time. According to place of birth females were 2½ times more sufferers in indoor thermal injuries and males were 4½ times more sufferers in outdoor thermal injury. 90% cases were below 30 years of age and 68% were children and women.

In this study it was significant to note that most of the cases i.e. 83% reached the hospital within 24 hours of accident irrespective of distance of site of fire accident from hospital. This signifies the amount of sufferings and anxiety of thermal injury. The 15% cases came after 48 hours of accidents irrespective of distance and percentage of burn.

In the present study 41 cases were studied after application of three types of dressing. Two of them were biological dressings (Amniotic membrane and Collagen sheet) and third was antibiotic gauze pieces. The amniotic membrane was applied over full burn area in 12 patients (Group A), one part of burn area 17 patient (Group C_1+E_1). Out of these 17 patients the remaining burn area was

treated with antibiotic gauze pieces in 3 cases (C_2) and with collagen sheet in 14 cases (E_2). This makes total of 29 surface areas where amniotic membranes were applied. The collagen sheet was applied over burn in 26 patients. Nine of them were the patient where it was applied over full burn area (B) and 17 (D_1+E_2) were the patients where it was applied over part of burn cases. The remaining part of burn surface was covered with amniotic membrane in 14 cases and with antibiotics gauze pieces in 3 cases (D_2). Thus making the total of 26 areas where collagen sheet was applied. A

Amniotic membrane is the inner one of two foetal membranes. It is thin, transparent, elastic and strong which can cover a wide surface area. It consists of five layers histologically. It is drieved from epiblast and is continuous with ectoderm of embryo. The collagen sheet is a product made from animal tissu rich in collagen such as serosa and sub mucus layers of caecum and intestines, pericardium and amniotic membrane. It is thicker than amniotic membrane. It is hazy, elastic and stronger. Each sheet is 6"x4" in measurement and rectangular in shape.

Pigeon (1960) described amniotic membrane like an extension of body skin and therefore a good substitute for autograft skin in superficial burn. Dino (1965) stated that amniotic membrane is a homograft that resembeles the skin being a direct continuation of foetal

integument along the umblical cord. The role of amniotic membrane has been evaluated experimentally as well as clinically. Sabella (1913) was the first person to use an amniotic membrane in the treatment of burn. Many workers found it useful as coverage material in superficial and deep burns. In deep burns it has to be changed from time to time. The collagen sheet has already been used with exteremely good results both in experimental and clinical studies by a number of surgeons in the country. As a dressing material it has already been used in different condition such as in traumatic wound with skin loss, infected and uninfected superficial or deep burn, donor area after skin grafting, experimentally produced raw area, in the posterior wall repair of hernia, as a mucosal cover in oral cavity and to gap the abdominal defects. Sinha et al (1972), Shanker (1975), Jain et al (1976) used collagen sheets as primary cover material in the management of burn. Later Srivastava (1978), Gupta et al (1978) found the collagen sheet as effective bio-dressing.

The availability, preservation and application of amniotic membrane are very simple and easy. It can be obtained fully prepared for application from clean labour rooms and obestetrics operation theatres at the time of child birth without any cost. It is, in intact form, attached with chorion and placenta and can be separated from them after washing out blood and mucoid material.

It can be applied freshly or can be preserved easily in sterile normal saline treated with 10 lac units of benzyl penicillin and 1 gram of steptomycin sulphate upto 1 month in refrigerator. Although it was discarded after 1 month of the preservation but there were no sign of disintigration. The application of amniotic membrane is also very simple. It can be applied over burn surface after debriding it in surgical O.P.D. or indoor wards under sedation. It can be left open or can be dressed for 12 hours to retain it on wound surface.

Availability and preparation is not that simple in the case of collagen sheet though attempts are now being made for the transfer of knowhow to a suitable intrepreneur for its commercialisation. It is costing Rs. 5/- for one sheet of 6"x4" size. It is prepared from collagen in orgens of slaughtered animals after combersome processing in Central Leather Research Institute Adyar, Madras. It is preserved in glass ampoules in preserving fluid containing ethylene oxide for years. The application of collagen sheet is simple and can be done in surgical O.P.D. and indoor wards under sedation after gentle debridment of wound. The dressings to cover and retain the sheet, were applied in every case.

It has been shown that the bacterial growth decreases most effectively when the temporary bio-dressing 'take up' on granulating wound. But in

superficial burn vascularization and 'take up' is not desirable and it is hoped that the more dense, uniform cuboidal surface of amnion discourages the penetration of fibroblasts. Graham, using the amnion alone for partial thickness defect, has shown that initial 'take' does not occur. The tough collagen sheet also discourages the penetration of fibroblast and capillaries.

In the present study it has been noted that pain disappeared in all 26 'take up' areas out of total 29 areas. Prabably coverage of exposed nerve endings by the membrane is responsible for disappearance of pain. Out of 3 cases where pain persisted after amniotic membrane application 2 were grossly contaminated at the time of admission and got infection afterwards. These findings are consistent with the published reports of other workers.

In the collagen sheet applied areas pain persisted for different period, in 24 out of 26 areas though all of them were 'take up ' cases. Probably the irritation of exposed nerve ending by rough collagen sheet and remaining preservative i.e. ethylene oxide on the surface of collagen sheet, were responsible for it. It seems that despite the washing of collagen sheet with normal saline preservative is not washed out fully. So 8 cases complained of increased pain and burning sensation.

It was noted that amniotic membrane adhered to raw surface within 6-18 hours in all the 26 'take up' areas while all the collagen sheet applied areas adhered within 12 - 18 hours. Adherence has been proposed to be the most important property of biological and synthetic dressings over deepithelized surfaces. It has also been shown that adherance of dressing to granulating or freshly produced wound can significantly reduce the bacterial contamination. As soon as amniotic membrane and/or collagen sheet adhered to surface patient was able to move that part. Most of the prosthesis and graft rely on the endogenous adhesive fibrin for adherence. This property of material is therefore determined by the strength of bonds that forms with fibrin. The studies have demonstrated that fibrin bonds preferably to collagen in normal skin.

Both the bio-dressing undergoes changes, as regards to their surface margins, thickness, dryness and colour. The wrinkles, though different in size and direction, develop in both the bio-dressings after 24-36 hours. The curling up of margins from the adjacent normal skin took place slightly earlier in collagen sheet (4-18 hours) than amniotic membrane (6-24 hours). The curling up from wound surface was also earlier in the case of collagen sheet (7-14 days) than that of amniotic membrane (9-14 days).

In most of the areas covered by bio-dressings, soakage was absent after their application . Out of 55 areas where bio-dressings were applied only 8 showed soakage and 5 of them were having pus. All the areas, treated with antibiotic gauze pieces, developed soakage and pus. Out of 29 amniotic membrane applied areas pus developed in 4 cases. 2 of them were having grossly contamination in their wounds at the time of admission and later on these wound surfaces rejected the membrane. These patients died later on due to shock and toxemia. Only one area out of 26 developed pus pockets underneath collagen sheet. None of the area rejected the collagen sheet. This rejection does not denote the rejection of foreign material by the surface but the formation of pus underneath bio-dressing, is responsible for it. In all the cases where pus was found the causative organism might have been present over wound surface prior to application of the bio-dressing. It showed that collagen sheet and amniotic membrane application prevents invasion of bacteria from out side.

Julian A Sterling (1956)used the amniotic membrane in old infected wounds. Martin (1972) in his experimental study concluded that amniotic membrane controls the bacterial population as allograft skin, however the decrease was greater than that with the skin

Exact mechanism is not known but different workers have suggested different mechanisms. Coloska and Suyder (1970) demonstrated multiple factors in human amniotic fluid which are said to be antibacterial Cholacho et al (1974) pointed out the uncertainity of remaining of these factor in amniotic membrane. Martin (1972) using in vitro technique reported the absence of such antibacterial factor. He proposed that in-vivo host's own defence mechanism deals with microorganism in biologically achieved close wounds with the help of bio-dressings.

Elhence et al (1978) noted that incidence of wound infection in collagen sheet treated patients was 50% as compared to 66.6% in autograft and 86% in antibiotics gauze piece treated wounds. This findings is not consistent with the finding of present study which showed only 8% infection in collagen sheet applied areas.

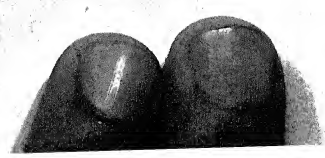
In this study the lower incidence of infection of collagen sheet treated cases than that of amniotic membrane treated cases, is not significant because 2 of the 4 infected membrane treated cases were grossly contaminated before the application. The collagen sheet is tested for sterilization before it is supplied while sterilization of amniotic membrane is not guranteed though it is preserved in proper disinfecting solution.

In present study out of 26 'take up' amniotic membrane applied areas 22 healed within 22 days completely and 4 partially which further took 31-35 days in complete healing while out of 26 collagen sheet applied areas 24 healed within 25 days with complete healing and 2 with partial healing which further took 25-35 days in complete healing. None of the 6 antibiotics gauze piece applied areas healed before 25 days. This shows that bio-dressing application enhances the process of healing and reduce the time taken in total healing.

Usually epithelization occured rapidly and completed in 14 days. Pigeon (1960) reported no evidence of reproduction of amniotic membrane. Dino (1965) stated that in grafted areas crust was formed which remained dry and free from infection. Then it peels off in 9-20 days. During first 5 days crust thickend gradually and changed its colour from serum yellow to brown. They also become corrugated, hard and tough. The findings of present study are in consistent with above mentioned study. Jain et al (1978) showed complete healing within 22 days in 80% of superficial burn in collagen sheet treated cases. He concluded that prevention of air borne infection after producing the close wound with the help of collagen sheet, was the main factor for early healing.

To compare the time taken by 2 biological

dressings it was noted that out of 26 'take up' membrane applied areas 3 healed within 17 days, 17 within 18-20 days and 5 within 20-25 days while in 26 sheet applied areas 14 healed within 15 days, 9 within 15-20 days and 4 within 20-25 days. This showed that the healing in more of collagen sheet applied areas was slightly earlier. This difference in time of healing is not significant because total time taken in the healing of superficial burn depends upon the number of cells preserved for re-epithilization. So it is the wound surface which determines the time of healing when sealed as close wound by bio-dressing. The difference in healing time between amniotic membrane and collagen sheet may be due to slower peeling off of amniotic membrane from the wound surface after healing has taken place under neath it than that of collagen sheet.



CONCLUSIONS

CONCLUSIONS

The comparative effects of amniotic membrane application and collagen sheet application were studied and compared in 41 cases of superficial burn. At the same time superiority of either of them was assesed.

This study was conducted in the patients of superficial burns of less than 50% of body area involvement who came to M.L.B. Medical College Jhansi. The conclusion drawn are as follows:-

1. Females are more sufferer in the age group of 15-25 years, while males suffers in the age groups of 0-10 years.
2. Amniotic membrane and collagen sheet provide good coverage to raw surface.
3. Both of them were easy to apply.
4. Both the bio-dressing effectively prevent the infection from out side.
5. Both the bio-dressing convert the open raw surface of burn area in to a close wound. Thus help the host defence mechanism to control infection. So both of them prevent the conversion of superficial burn to deeper degree of burn.
6. Exudation and soakage over the surface does not occur in the both cases. If it occured then it is due to the

already present microorganism over surface prior to application of amniotic membrane and collagen sheet.

7. Loss of fluid and protiens from the surface is decreased as the serum clots between the bio-dressing and raw surface forms a effective barrier for fluid and protien loss.

8.Both the bio-dressing were applied only once. So local management of the wound becomes very easy and economical (free of cost in the case of amniotic membrane and Rs.5/- for 6"x4" area in the case of collagen sheet.

9.The discomfort and suffering of the patient are considerabely reduced in both the amniotic membrane and collagen sheet applied cases.

10.The part covered with both the bio-dressing are mobilized as soon as they adhere to surface & active passive physio-therapy is easily accepted by patients.

11. The rate of healing of wounds, covered with two bio-dressings, was faster. This is because of prevention of extraneous infection, non disturbance and preservation of potent epithelizing cells.

12. The wounds where partial healing was observed, were deeper degree of burn.

13.No contracture was observed in any case covered with collagen sheet and/or amniotic membrane.

14.The scars of completely healed areas were pink with smooth surface and flat margin in both the cases.

On comparing the effects of two bio-dressings to assess the superiority of either of them, following conclusions were drawn -

1. Both the dressings, without any significant difference, are good covering material, very easy to be applied, effective in controlling extreneous infections and preventing fluid and protein loss.

2. Availability is no problem in the case of amniotic membrane while collagen sheet can only be available from Central Leather Research Institute Adyar, Madras.

3. The preservation of amniotic membrane is easy and cheap but for shorter duration while that of collagen sheet is more difficult, costlier but for longer duration.

4. Collagen sheet is supplied after undergoind the sterlization tests while sterile status of amniotic membrane is not guranteed.

5. Amniotic membrane is a natural bio-dressing without any cost. The collagen sheets are prepared after difficult processing and cost Rs. 5/- for each sheet.

6. A single Amniotic membrane can cover 8"x14" raw area while single collagen sheet can cover only 5½"x3½" area. Thus for 15% burn of adult a single amniotic membrane is sufficient while 6-7 collagen sheets are required in that case.

7. For larger wound area open wound is closed completely

and safely if amniotic membrane is applied while collagen sheet applicationshave risk of developing gap between two sheets exposing the area for infection when it became dry.

8. No dressing is needed to retain amniotic membrane in place initially while one or two initial dressings are needed to retain the collagen sheets in its place, till it becomes adhered.

9. Difference in infection rate is not significant.

10. The collagen sheet peeled off earlier than amniotic membrane and thus take slightly lesser time in healing.

11. Pain relieved atonce when amniotic membrane is applied while pain is not relieved or increased at times when collagen sheet is applied. So amniotic membrane decidedly better in this respect.

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